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CHARACTERIZATION OF AN ACID-STABLE PEPTIDE HYDROLASE AND OF PEPTIDE HYDROLASE A IN GERMINATED WHEAT

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A~traet--Peptide hydrolase A from germinated wheat has been separated from an acid-stable peptide hydrolase. The stability of these enzymes to pH and temperature and their K~, values are described. Some peptides hydrolyzed by the acid-stable enzyme are reported.

INTRODUCTION

PP,EVIOUS work 1'2 has shown that germinated wheat contains an enzyme which was not adsorbed on carboxymethyl cellulose (CMC) and which hydrolyses alpha-N-benzoyl-L-arginine ethyl ester (BAEE). This enzyme was resistant to low pH and elevated temperatures and was distinct from peptide hydrolase A (PHA). This report describes its purification and characterization and the characterization of the PHA enzyme free of the acid-stable enzyme.

RESULTS AND DISCUSSION

The separations obtained by CMC chromatography and Sephadex G-100 filtration are shown in Figs. 1 (for CMC treatment), 2 and 3 (for G-100 filtration). When the unadsorbed enzymes from *CMC* (fractions 5-14, Fig. I) were applied to G-100, the separation shown in Fig. 2 was obtained. Fractions 26-35 (Fig. 2) contained mainly PHA and fractions 19-25 mainly the acid-stable BAEE-ase. Fractions 26--35 when re-applied to G-100 gave the elution pattern shown in Fig. 3. Fractions 32-45 of Fig. 3 were pooled for the PHA, which hydrolyzes both substrates. Since the assay with BAEE is much less sensitive than the one with alpha-N-benzoyl-DL-arginine-p-nitroanilide (BAPA), the former activity is often not evident until fractions have been concentrated.

Table 1 shows the purification accomplished by these procedures. The ratio of PHA activities with the BAEE and BAPA substrates was approximately 2. This is in reasonable agreement with this ratio for the corresponding enzyme from barley, 3 whereas the acid-stable BAEE-ase (fractions 19-25, Fig. 2) has very little activity with BAPA.

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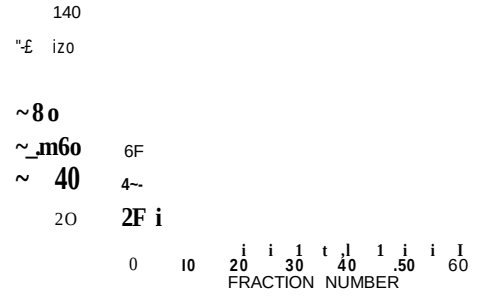

FIG. 1. CMC CHROMATOGRAPHY OF GERMINATED WHEAT EXTRACT.

- - o.d, 280 nm; •----O PHA with BAPA **as substrate;** o-- -e PHA **and acid-stable** BAEE-as¢ **with BAEE as substrate.**

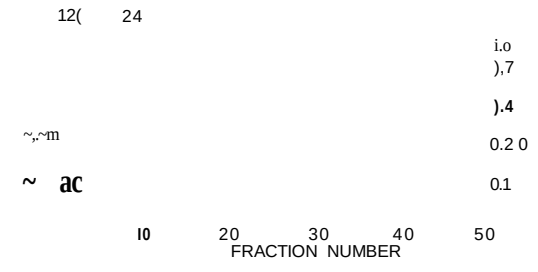

FIG. 2. SEPHADEX G-100 FILTRATION OF PROTEINS UNADSORBED ON CARBOXYMETHYL CELLULOSE.

- - o.d. 280 nm; • • PHA with BAPA **as substrate;** • .... • PHA **and acid-stable** BAEE-ase **with BAEE as substrate.**

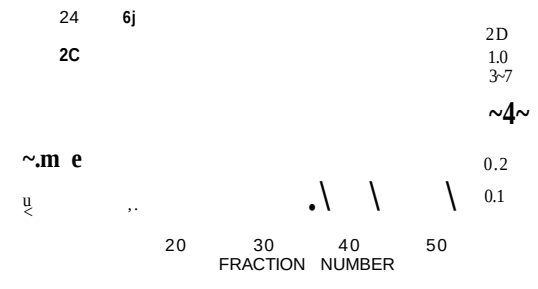

FIG. 3. SEPHADEX G-100 FILTRATION O12FRACTIONS 26-35 FIG. 2.

---- o.d. 280 rim; • • PHA with BAPA **as substrate;** • .... • PHA **and acid-stable** BAEE-ase **with BAEE as substrate.**

Dialysis of the acid-stable BAEE-ase (fractions 19-25, Fig. 2) and the PHA preparations (fractions 32-45, Fig. 3) against 0.05 M tartrate, pH 3.5, gave 80 per cent and 0 per cent recoveries, respectively, of activity with BAEE substrate. Thus, the wheat PHA is similar to barley PHA in this respect. The pH optimum for the acid-stable enzyme with BAEE substrate is approximately pH 7. A variety of simple peptides were hydrolyzed to various degrees by this enzyme at pH 7 (Table 2). Tests with hemoglobin substrate at pH 3.8 and casein at pH 5.5 revealed no hydrolysis. The data are consistent with the enzyme being a peptide hydrolase of a low order of specificity.

TABLE 1. PURIFICATION OF PHA AND ACID-STABLE BAEE-AS~

Purification	Vol. (ml)	Protein (mg/ml)	Activity (units/ml) r" BAPA	Activity (units/ml) BAEE'	Recovery (~) "BAPA	Recovery (~) BAEE"	Specific activity units/mg (Protein) * 'BAPA	Specific activity units/mg (Protein) * BAEE '	Ratio specific activity BAEE BAPA
Carboxymethyl cellulose									
Sample applied:									
Extract dialyzed vs. 0'005 M acetate	30	17.4	125	990	100	100	7	57	8
Products:									
Unadsorbed hydrolases (fractions 5-12, Fig. 1)	27	5.1	92	1330	69	120	18	262	14
1st G-100 filtration									
Sample applied:									
Unadsorbed hydrolase~ (fractions 5-12, Fig. 1)	24	5.1	102	1460	100	100	20	287	14
Products:									
Acid-stable BAEE-ase (fractions 19--25, Fig. 2)	31	0.5	9	590	11	52	18	1180	66
PHA (fractions 26-35, Fig. 2)	14	1'8	144	590	83	43	80	330	4
2nd G-100 filtration									
Sample applied:									
PHA (fractions 26-35, Fig. 2)	21-5	1.7	94	430	100	100	55	250	4
Products:									
Acid-stable BAEE-ase (fractions 23-31, Fig. 3)	13	0'39	31	415	20	58	80	1060	14
PHS (fractions 32-45, Fig. 3)	14"5	0'68	130	265	95	41	191	390	2.0

TABLE 2. HYDROLYSIS OF PEPTIDE AND ESTER SUBSTRATES BY THE ACID-STABLE ENZYME*

Ala-asn	--	Gly-tyr	-I-
Ala-gly-gly	-I- slow	Hippuryl-arg	--
Ala-lys	q- complete	Hippuryl-gly	--
Ala-phe	-t- complete	Leu-gly	q-
Ala-val	q- complete	Leu-gly-gly	-1- complete
Gly-glu	--	Lys-ala	i
Gly-gly	q-	Lys-gly	-I- slow
Gly-gly-gly	+	Lys-lys	q-
Gly-leu	q-	Phe-leu	q- complete
Gly-lys	q- complete	Trp-gly	q-
Gly-phe	-t-	Trp-leu	q- complete
Gly-thr	-/- slow	Tyr-gly	.t.
Gly-trp	q-	Tyr ethyl ester	q- complete

*+ complete=complete hydrolysis of the quantity of substrate used; + slow=very little hydrolysis after 16 hr incubation.

The Km for PHA (Table 3) suggests that this enzyme is a less active one than the corresponding one from Trophy barley 3 which has Kin=4"4 × 10-5 M, but is close to that for the PHA of wheat embryo, 4 i.e. 1.0 x 10-4 to 1.3 x 10-4 M. The Km of the acid-stable BAEE-ase (Table 3) is about the same as that for peptide hydrolase B from Trophy barley. 2 The thermal inactivations of the acid-stable enzyme and PHA are shown in Table 4. The wheat PHA changes with temperature as did barley PHA treated similarly. 3 The acid-stable enzyme is relatively resistant to denaturation by heat.

EXPERIMENTAL

Material and Methods

Plant material. The wheat *(Triticum aestivum* L.) was a mixture of hard red spring varieties grown at Madison, Wisconsin, in 1963. It was stored at --25° until used.

4 N. PRENTICE, W. C. BURGER, J. KASTENSCHMIDT and M. MOELLER, *Phytochem.*, to be published.

TABLE 3. KINETIC CONSTANTS WITH BAEE SUBSTRATE

	PHA*	Acid-stable BAEE-aset
K_m, M	1"3 x 10-4	2-9 × 10-4
V, Units/0.1 ml	29	91

* Fractions 32-45, Fig, 3.
t Fractions 19-25, Fig. 2.

TABLE 4. HEAT INACTIVATION OF PHA AND ACID-STABLE BAEE-ASE

	% Activity remaining	
Temp.	PHA	Acid-stable BAEE-ase
35	92	101
45	34	104
35	34	94

0"1 to 0'2 ml samples of enzyme were heated at indicated temperatures for 1 hr in 0"1 M succinate, 0.2 M NaCI, and 0.004 M EDTA, pH 5.9, in 8 x 75 mm closed test-tubes.

Germination and Extraction of Wheat; Assays for Protein and Peptide Hydrolase Activity; Moisture Determination; Treatment of Enzymes at Various pH's and Temperatures; Determination of Kinetic Constants; Reaction with Peptide Substrates

These procedures have been described previously,[z,3]

Purification with CMC and Sephadex G-IO0; Determination of pH Optimum

These materials and operations have been described.[4,5]

Endopeptidase Assays

The methods for endopeptidase with hemoglobin and casein substrates have been described.[6]

W. C. BURGER, N. PRENTICE, M. MOELLER and J. KASTENSCnMIDT, *Phytochem.*, to be published.
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